Toxicology and safety assessment in DDD

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Charlotte Nilsson

- B.Sc., biology (+chemistry), SU
- M.Sc., toxicology, KI
- Ph.D., toxicology, KI

- Risk assessment, IMM, KI
- Toxicologist, AstraZeneca
- Education coordinator, IMM, KI
- Education coordinator etc, Swetox

From Jan 1, 2019:

- Project leader at RI.SE

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Toxicology and safety assessment in drug discovery and development

First part:

- Tox and safety in DDD for small molecules

Second part:

- Tox and safety for biopharmaceuticals

Discussions and questions throughout!

Questions or clarifications afterwards: charlotte.nilsson@swetox.se

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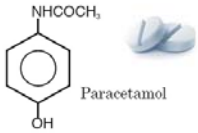
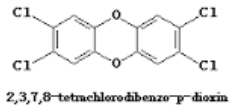
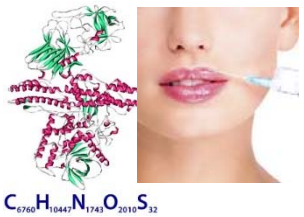
Toxic?

Water Table salt ”Alvedon” Bitter almonds Dioxin Botox







Paracelsus (1493-1541)

”What is that is not poison?
Everything is poison.
The dose alone makes
something a poison.”

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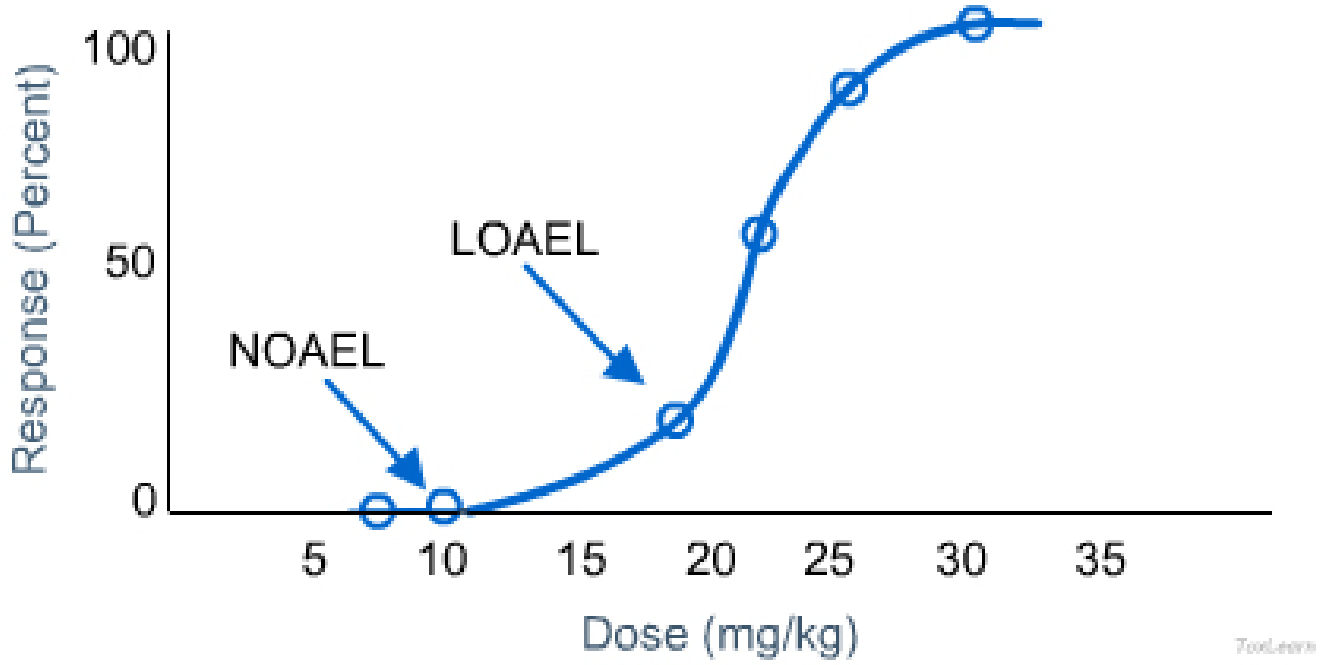
Dose response curve



LOEL = Lowest Observed Effect Level

NOEL = No Observed Effect Level

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Time: not only for how long, but when…

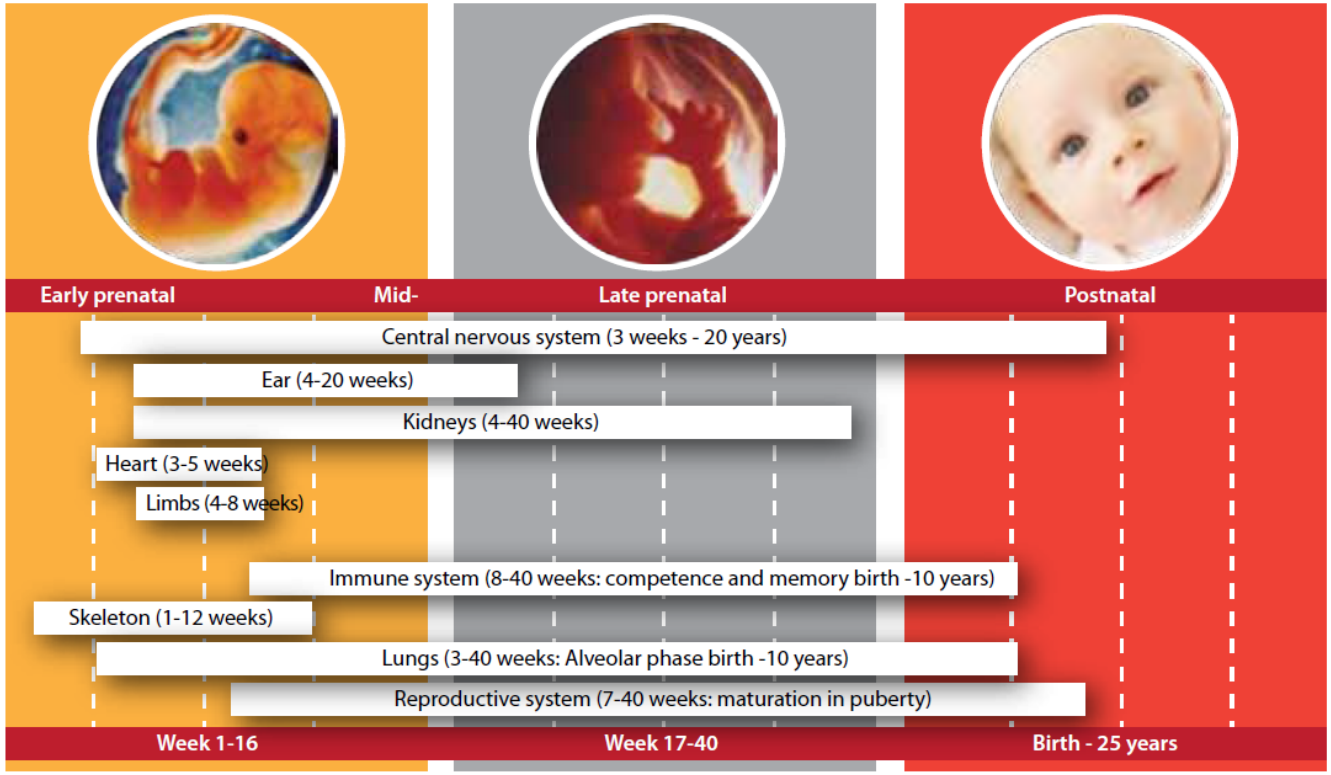

Figure 1.4. Timing of organ development. Hormones affect each of these indicating that they are important, and in different ways, throughout life.

WHO/UNEP EDC report 2012

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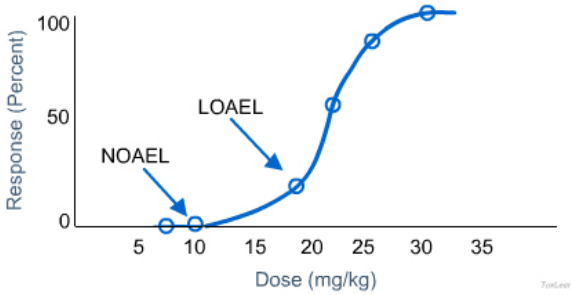
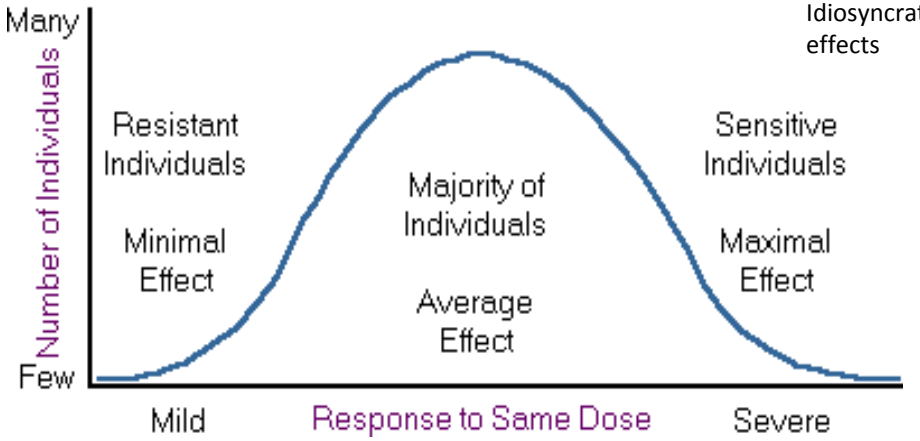
”Cornerstones of Toxicology”; Hayes & Dixon 2017



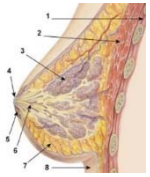
Main messages:

- Dose matters (and time)
- People matter

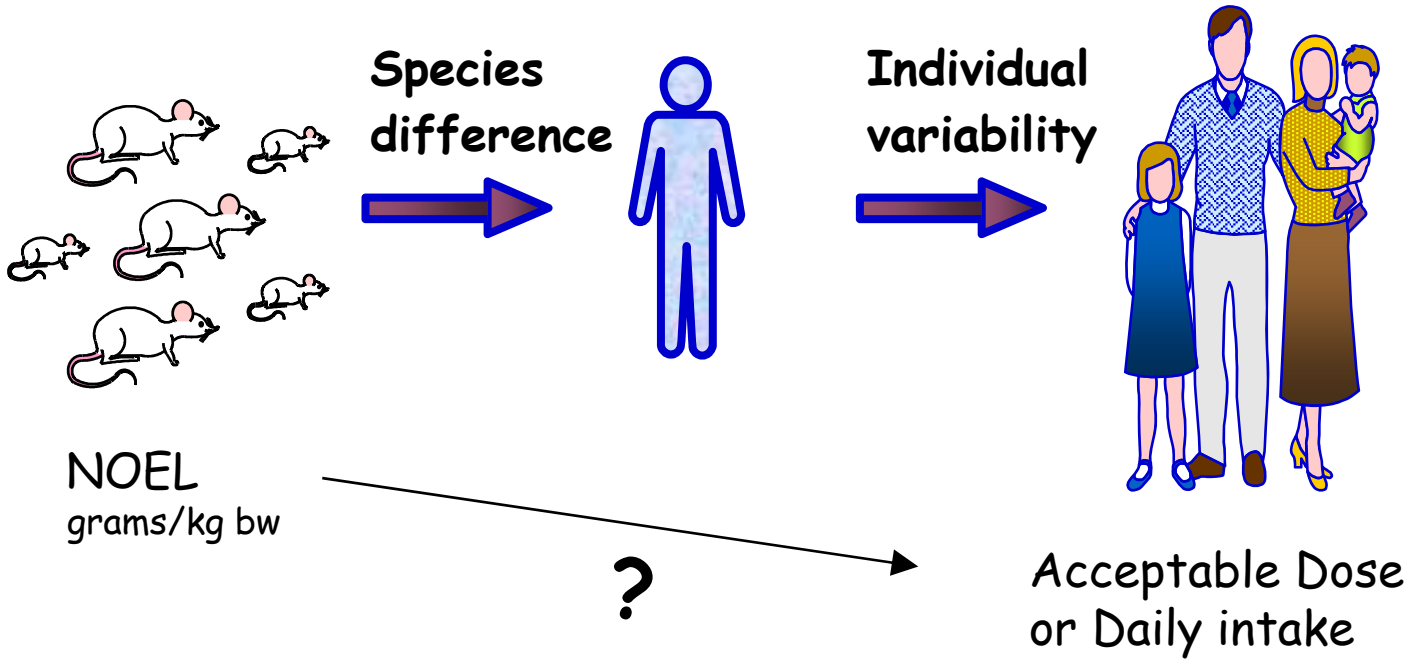


Iressa. Idiosyncratic effects

- Things change (= the body transforms the chemical)

Extrapolation from animal to man



Risk assessment

Dioxins and PCBs
in organic eggs!!!

Which eggs do you choose in the store?
“Regular eggs” OR “Organic (eko) eggs”

Do you choose differently due to the article?

START | NYHETER

Giftlarm om ekoägg

Av **TT**
Publicerad: 12 October 2016, 18:35

Halterna av miljögifterna dioxin och PCB har ökat i ekologiska ägg. Livsmedelsverket konstaterar att halterna är högre än i andra ägg.

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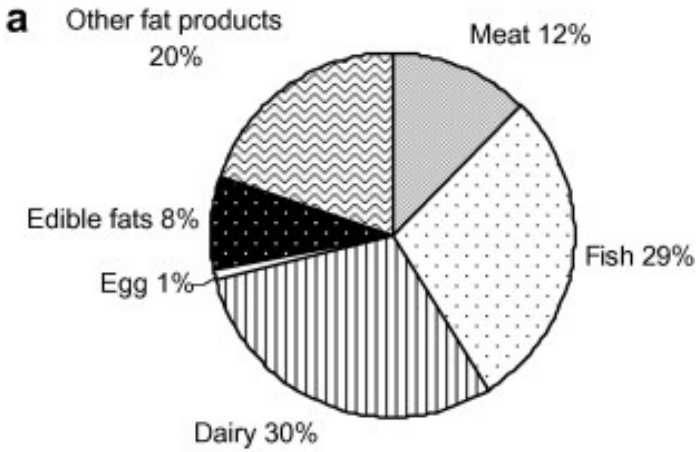
Total intake of dioxin and PCB



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Levels in mother's milk > organic eggs…

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The DDD process

”If it is efficacy data we want to believe it, but if it is toxicity data we want to explain it away”….

It’s all about
EFFICACY & SAFETY!

Purpose of Toxicity testing and Safety Assessment:

- Find and characterize the adverse effects
  - Identify e.g. biomarkers
- Guide selection of safe dose to humans

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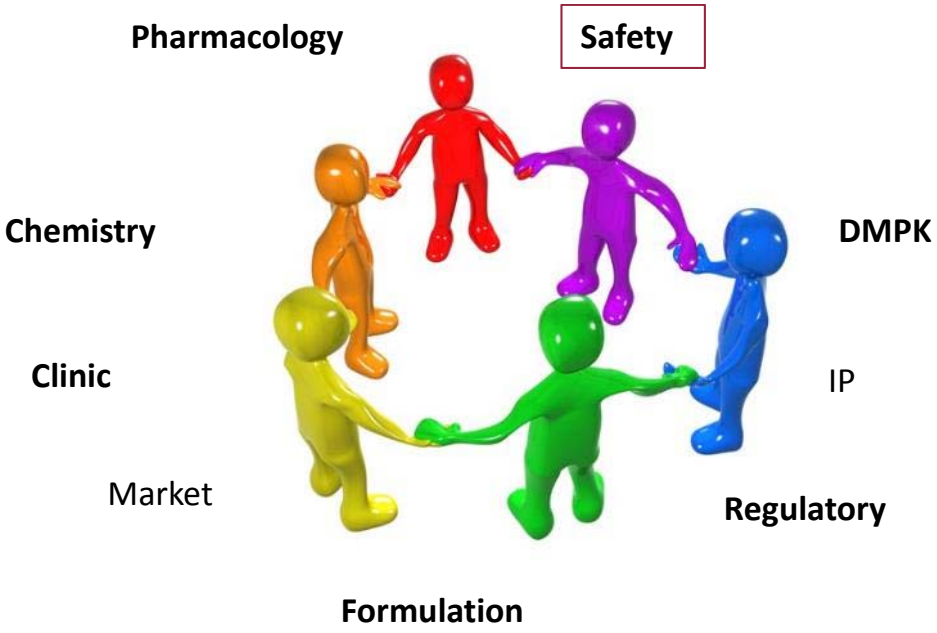
The different functions/areas



”What about side effects?
We have to check if this enzyme is doing something important in the brain.
Is it also present somewhere else in the body?
Can the molecules also affect other enzymes, etc?”

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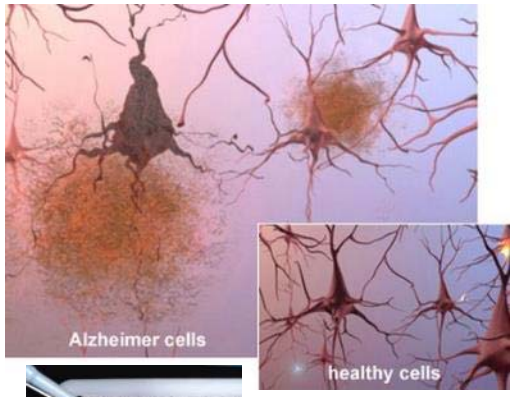
The different phases

Discovery

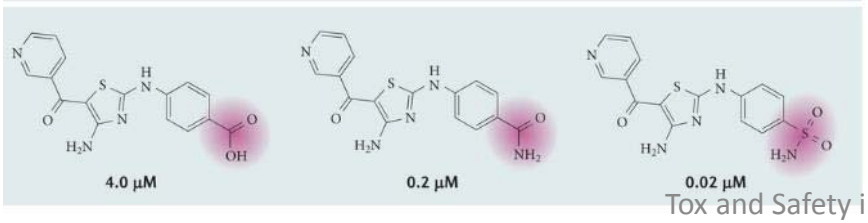
Development

Launch

Target ID and Selection | Hit ID and Selection | Hit to Lead | Lead Optimization | Pre-Clinical Development | Phase I | Phase II | Phase III | Approval Market



Candidate Drug



Nature Reviews | Drug Discovery

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Not many drugs reach the market…

OPINION

Can the pharmaceutical industry reduce attrition rates?

Ismail Kola and John Landis

2004

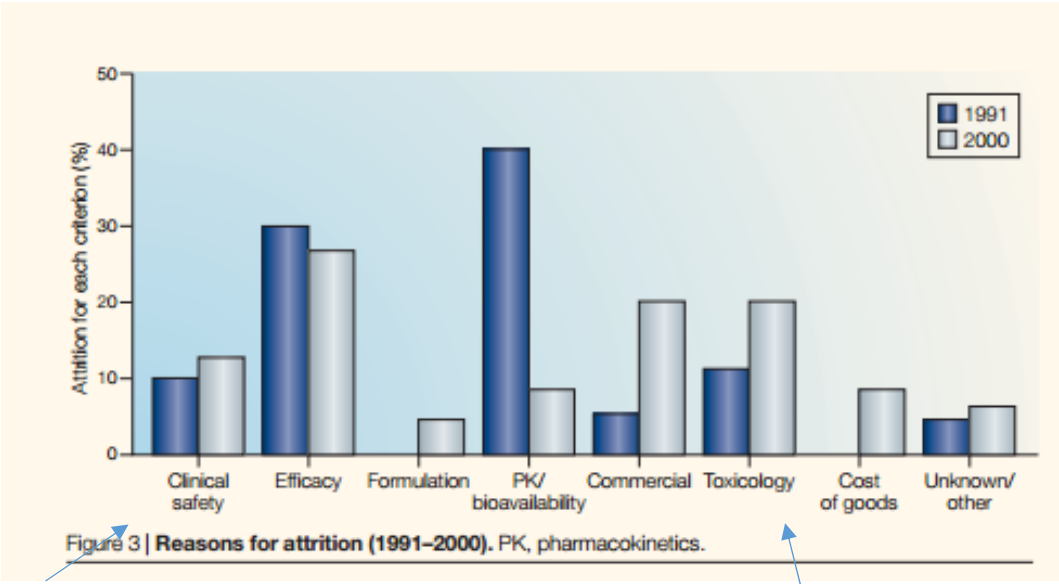

Figure 3 | **Reasons for attrition (1991–2000).** PK, pharmacokinetics.

An analysis of the attrition of drug candidates from four major pharmaceutical companies

Michael J. Waring[1], John Arrowsmith[2], Andrew R. Leach[3], Paul D. Leeson[3,4], Sam Mandrell[5], Robert M. Owen[6], Garry Pairaudeau[1], William D. Pennie[6,7], Stephen D. Pickett[1], Jibo Wang[8], Owen Wallace[8,9] and Alex Weir[2]

2015

Termination reason	Overall
Clinical safety	68 (11%)
Commercial	40 (7%)
Efficacy	55 (9%)
Formulation	9 (1%)
Non-clinical toxicology	240 (40%)
Patent issue	1 (0.2%)
Pharmacokinetics or bioavailability	29 (5%)
Rationalization of company portfolio	124 (21%)
Regulatory	2 (0.3%)
Scientific	33 (5%)
Technical	3 (1%)
Other	1 (0.2%)
Total	605

Year 2000-2010

It's all about
EFFICACY & SAFETY!

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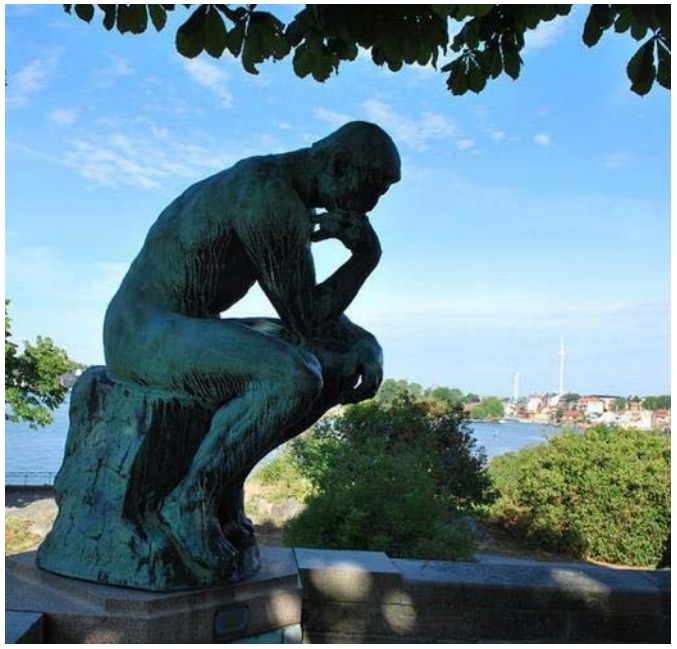
”Once the compound is in the vial, all the properties are fixed. All that remains are for them to be discovered.”

To that, add various aspects of **risk**:
Risk assessment, risk perception,
risk acceptance, risk communication,
etc, etc…



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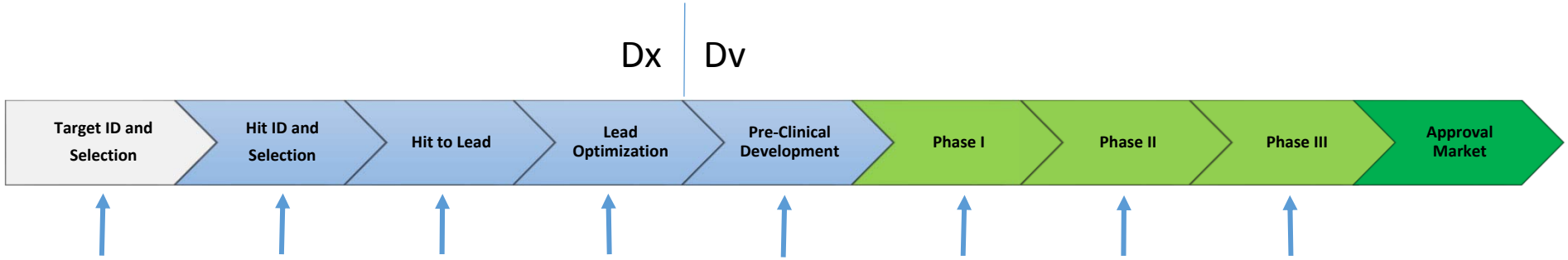
Safety in DDD



Target-related vs. **chemistry**-related toxicity

Tools: in silico, in vitro, in vivo

Margin of safety!

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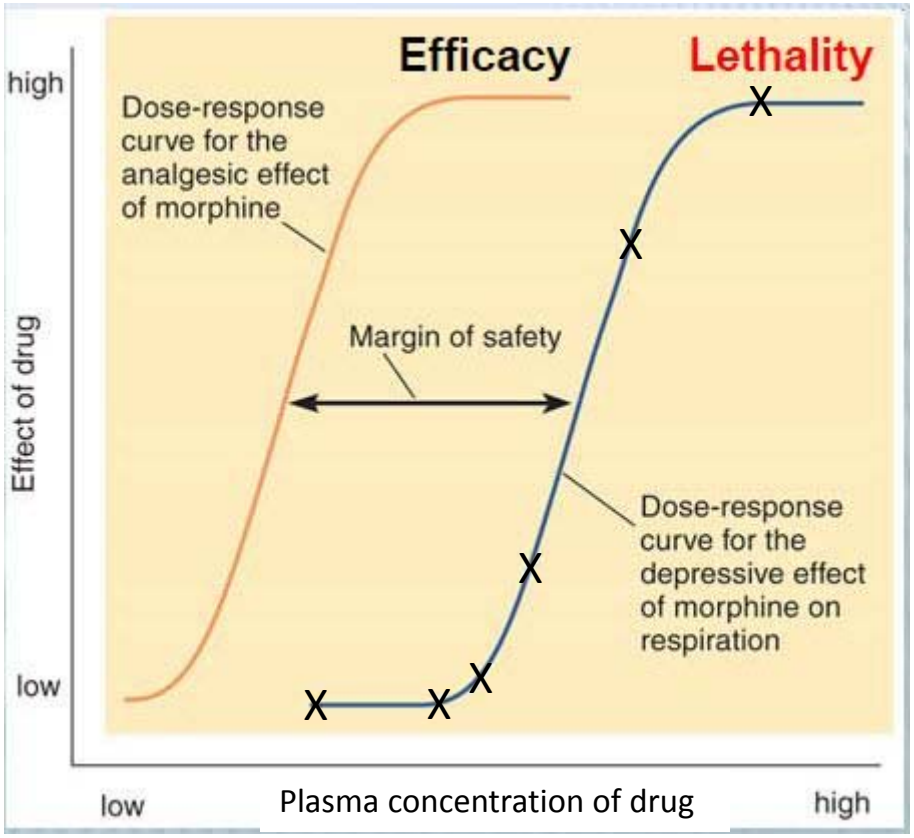
Margin of safety



New data -> curves may shift (Efficacy curve usually shift to the right….

Judgement calls re **which toxic effects are critical in humans**, and when they start to appear! (**NOAEL**, **LOAEL**)

Target-related toxicity

and onwards

- On-target (pharmacological) effect too strong / unacceptable
  - Target organs (efficacy vs safety)
  - A sufficient safety margin?
  - Toxicity found in animals does not translate into humans?

- Off-target effects (affects related/other known targets)
  - Secondary Pharmacology
  - Screen (chemical series/lead compounds) in silico and in vitro

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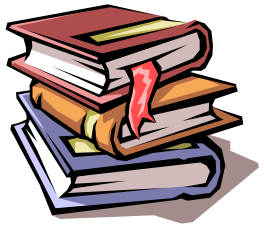
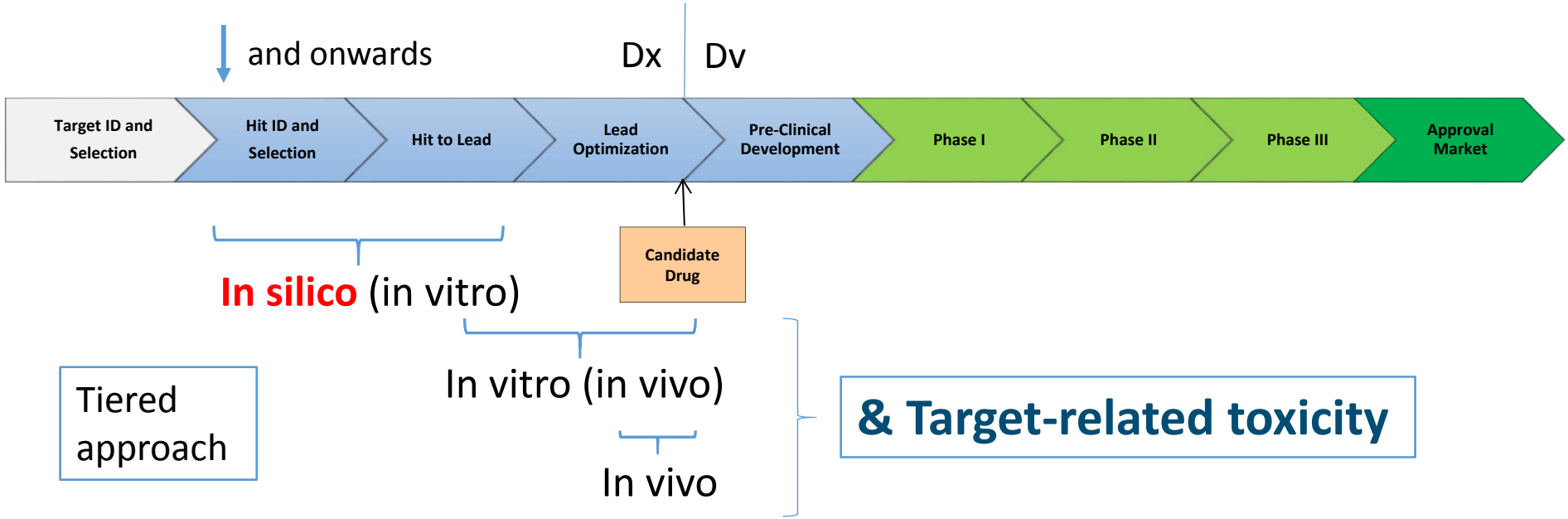
Target safety assessment

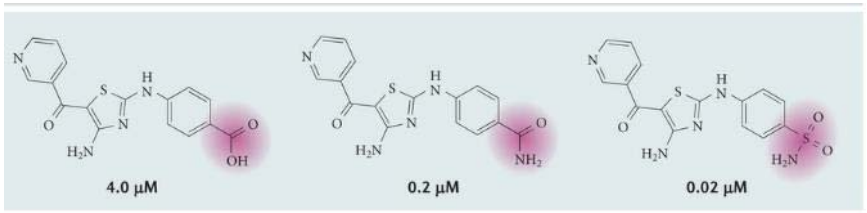


- Biological role of the target
- Tissue expression pattern
- Selectivity issues (biological role & expression)
- Phenotype caused by mutations in animals and humans.
- Pre-clinical or clinical safety data

- Consider also: indication, duration of treatment, patient population (age, sex, WOCBP, co-morbidities), possible biomarkers
- Acceptable risk profile?
- Plan forward! Collaborate with other functions!

Chemistry-related toxicity





Nature Reviews | Drug Discovery

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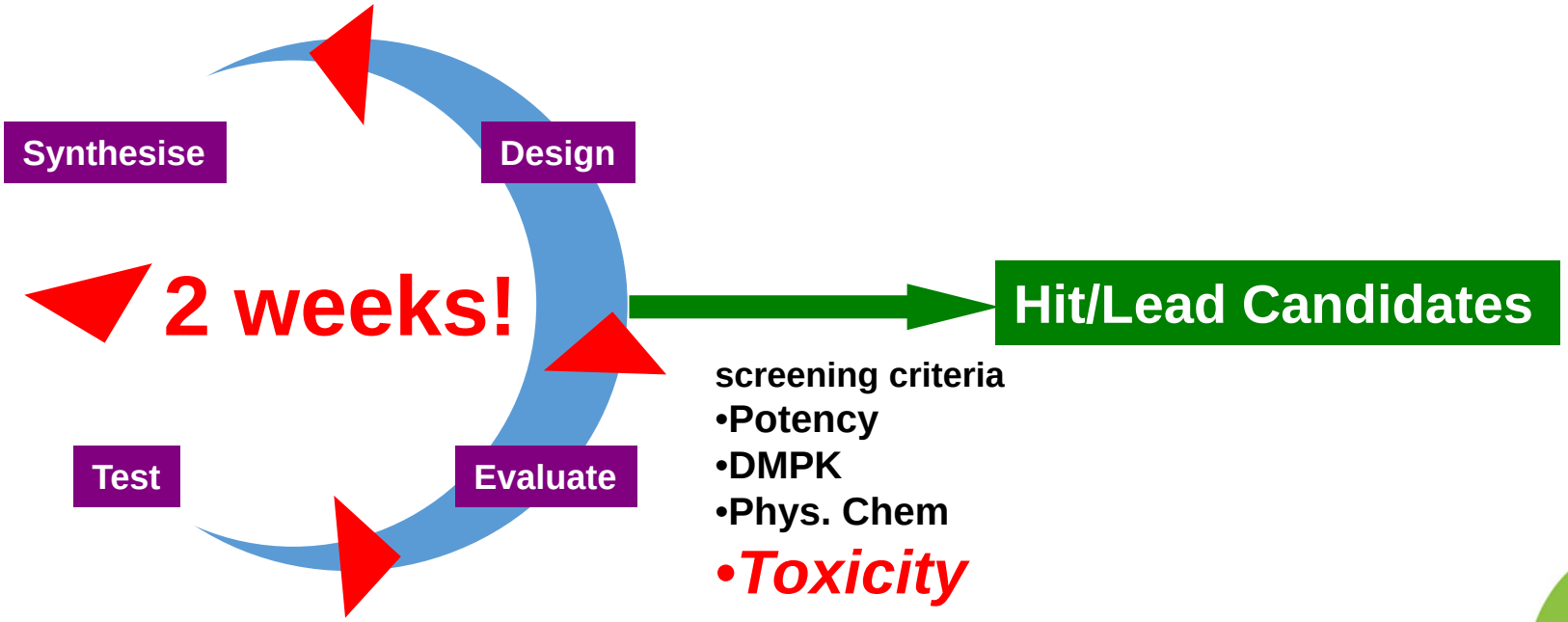
Collaborations during Hit and Lead phases

Drug Discovery



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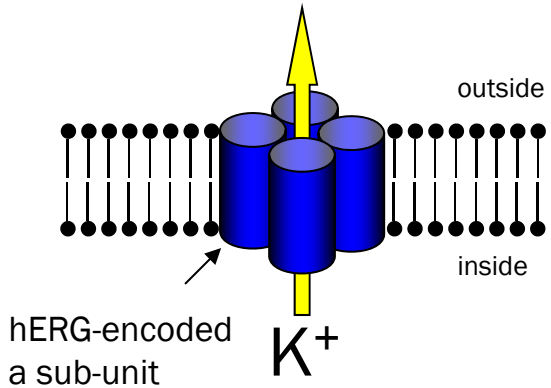
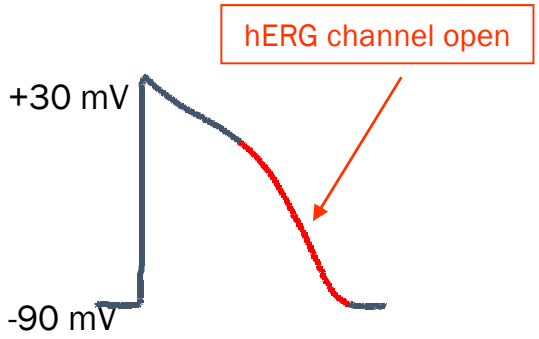
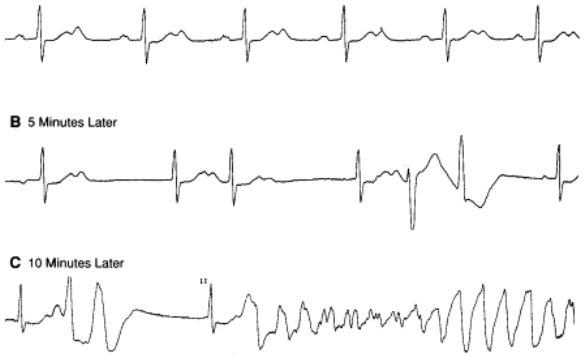
Example of a frontloaded screen: hERG

- hERG: human ether-a-go-go-related gene.
- An ion channel expressed in cardiac cells
- Outward flow of potassium ions through the hERG channel is one of the main currents responsible for the later repolarisation of the cell.





Cardiac action potential (AP)



- **Blocking of the hERG channel:** increases AP duriation and QT interval, can lead to fatal arrhythmia (Torsade de Point)

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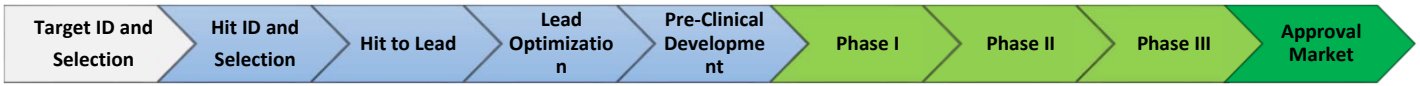
hERG and drug discovery

Amount of cmpd
Not rats
Predicted Cmax…



- In silico screenings – as early as possible
- In vitro screenings – LI/LO
  - Activity IC_{50} preferably below 10 µM
  - Activity IC_{50} 30-100x higher than predicted human $C_{max(free)}$
- In vivo tests – LO/prenomination/post-CD selection
  - Guinea pig MAPD90 test
  - Dog telemetry (measuring CV parameters)
  - No changes at 30-100x higher than predicted human $C_{max(free)}$
  - Criteria may differ for life-threatening diseases

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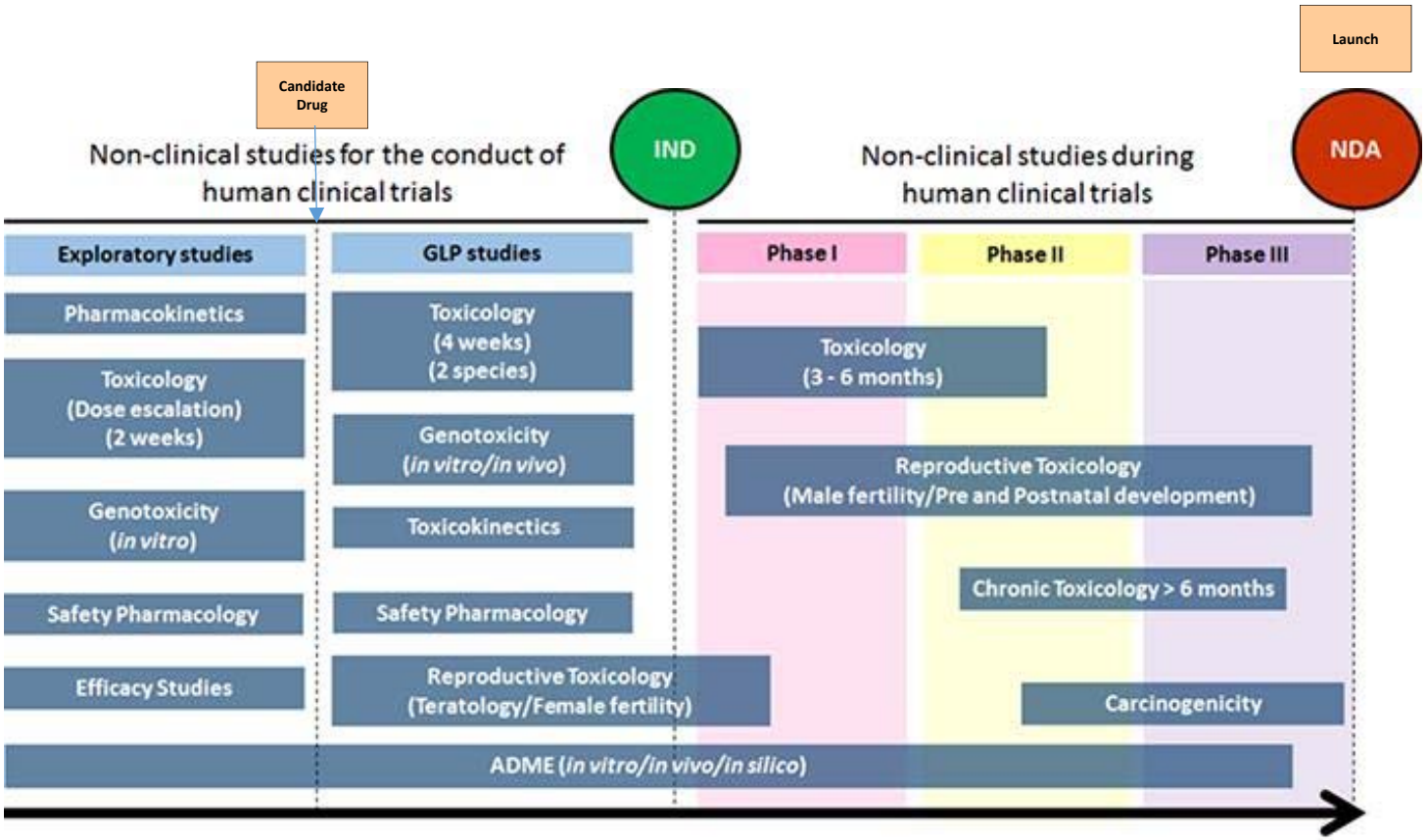
Safety during DDD – lab work



GLP = Good Laboratory Practice – generating high-quality, trackable data

IND = Investigational New Drug – for going into clinical studies

NDA = New Drug Application – for beginning to sell the drug on the market

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Safety in the Drug Discovery phase and beyond

- Continuous re-evaluation of the risk profile
  - Changes in margin of safety?
  - Use any available feedback!
    - From earlier CDs in-house; from competitors
- Prepare for Development and Clinical studies, eg:
  - suitable tox species?
  - tolerabe dose levels? (tox & FTIM)
- Collect tools for the future
  - endpoints, assays, biomarkers
- Avoid surprises in the 1-month tox study!
  - And of course in the clinic….

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Safety in Development phase

- Regulatory toxicology, according to Guidelines
  - Non-standard studies can be added
  - Two species, rodent and non-rodent (rat&dog)
- GLP!

- Support upcoming clinical studies
- Provide data for registration

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Alternatives?

- 3R
  - Replace animal models
  - Reduce the number of animals
  - Refine the experimental procedures to minimize suffering and increase value

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REPLACE animal models: from rabbits to modern immunologic techniques

Season 4 episode of Mad Men, Roger Sterling asks Joan Harris, "Did you take a rabbit test?"

Joan, played by Christina Hendricks, deserves better than Roger's shoddy treatment. Photograph: BBC/AMC/Lionsgate/Frank Ockenfels 3 BBC/AMC/Lionsgate/Frank Ockenfels 3/AMC/Lionsgate

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REDUCTION

- method development for blood sampling

Drug safety study in rats: BEFORE

No of animals	Samples per animal	Volume
SAFETY STUDY		
100		
EXTRA FOR BLOOD SAMPLING		
36	5	500 µl
TOTAL		
136		

AFTER

No of animals	Samples per animal	Volume
SAFETY STUDY		
100	2	25 µl
~~EXTRA FOR BLOOD SAMPLING~~		
~~36~~	~~5~~	~~500 µl~~
TOTAL		
~~**136**~~ **100**		

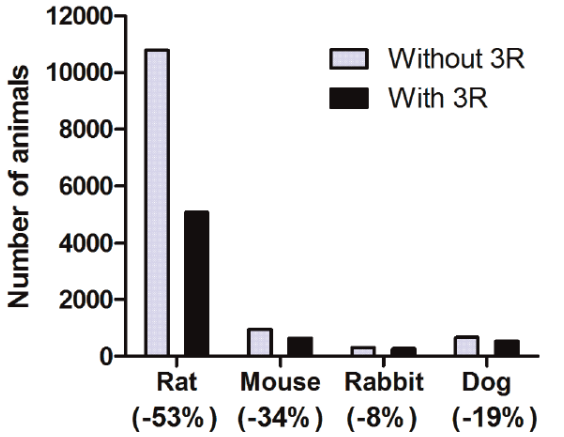

Figure 3. Estimated reduction in number of animals used in toxicity studies at Safety Assessment Research Unit. Actual number of animals used in 2009 (black bars) compared with the estimated number of animals if no reduction projects would have been implemented (grey bars).

Data från Jonsson et al., 2012 "Capillary microsampling of 25 µl blood for the determination of toxicokinetic parameters in regulatory studies in animals." Bioanalysis. 2012 Mar;4(6):661-74

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REFINE the experimental procedures

- animals (almost always) thrive in company

Group housing of male mice

- Calmer animals (-> better science!)
- Better work environment
- Lower costs

Data från Annas et al., 2013 "Group housing of male CD1 mice – reflections from toxicity studies" Laboratory Animals 2013, 47:127-129

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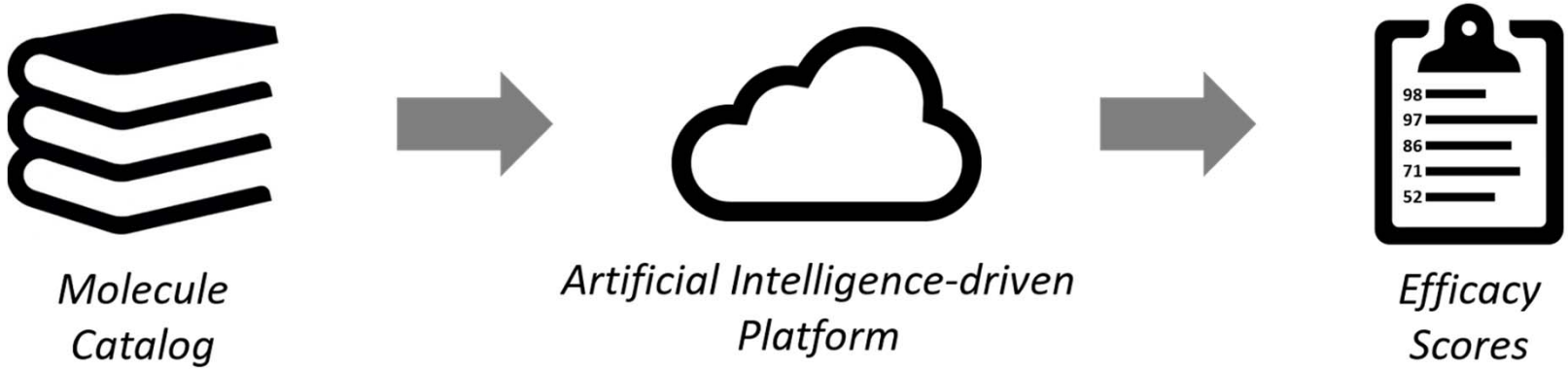
AI in Drug Development



Molecule Catalog

Artificial Intelligence-driven Platform

Efficacy Scores

and/or
Safety!

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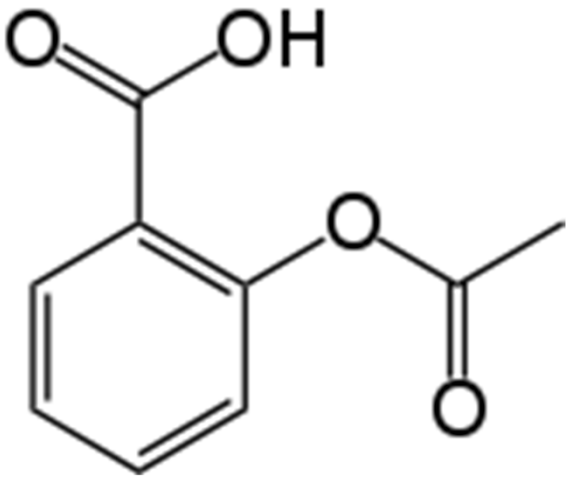
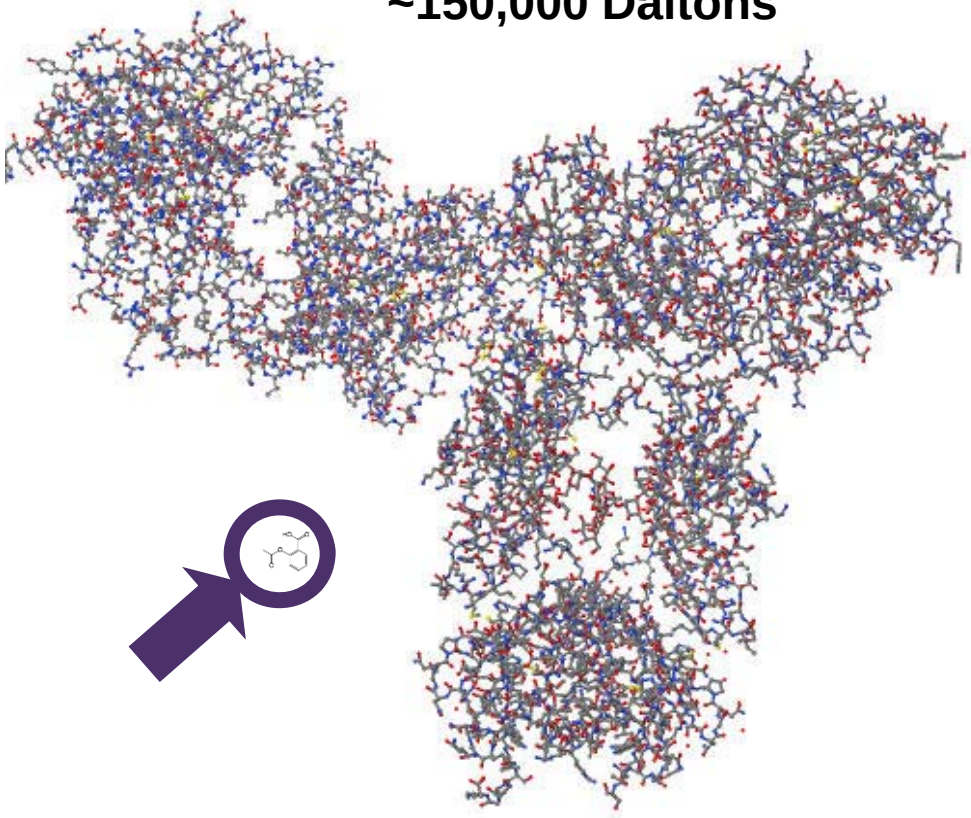
Now over to biologicals



Aspirin (acetylsalicylic acid)
180 Daltons

Human IgG1
~150,000 Daltons



Small molecules v.s. biologicals

Small molecules

- Small!
- Species-independent
- Short-acting
- Metabolized
- Safety concern: toxicology (target- and/or chemistry-related)

Biologicals

- Big!
- Species-specific
- Longer-acting
- Degraded
- Immunogenic
- Safety concerns: exaggerated pharmacology (target), immune response

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No need for....

- Metabolism studies
  - Not relevant (degraded, not metabolized)
- Genotoxicity studies
  - Interactions with DNA unlikely
- Carcinogenicity/chronic toxicity studies
  - Difficult due to eventual immunogenicity
  - May be irrelevant due to no activity on eg rodent target
  - An issue, with more and more chronic treatments
  - While not genotoxic, could drive neoplasm growth

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Exaggerated pharmacology

- I.e., target-mediated toxicity
- Depends on target and mechanism of action
  - Same idea & approach as for small molecules
  - Tested in a similar way
- Critical: animal species for tox testing must be relevant from a target perspective
  - ”most relevant”: often the non-human primates…
  - Transgenic animals (humanized mice) may be an option

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Immunogenicity (anti-drug)

- All proteins may be recognized as foreign
  - The hypervariable region of a new ab = foreign
  - If the foreign protein is large enough: immune reaction
- Induction of antibodies against drug: anti-drug ab (ADA)
  - Can vary from low-titer, low-affinity, transient ADAs to high-titer, high affinity ones.
  - The end result of the immune response can vary from none to life-threatening…
- Even ab drugs that are homologues of human proteins can be immunogenic
  - Often related to **manufacturing process** rather than protein sequence!

Immunogenicity (ADAs)

- Binding antibodies (ADA):
  - Increases or decreases t½ of drug
  - Changed PK/PD
- Neutralizing antibodies (Nab):
  - Blocking of target binding
  - No (desired) clinical effect
- Cross-reactive ADAs:
  - If drug is identical to endogenous protein: -> autoimmunity (= immunotoxicity)

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Immunotoxicity – too much/little

- Immunosuppression
  - Inhibition of the immune response, can lead to e.g. susceptibility to infections
  - Can be caused by a blocking ab without effector fxn
- Activation of the immune system
  - Drug acts as immunogen
  - Hypersensitivity
  - Autoimmunity
  - Ab with effector fxn
    - Which cells are activated? Cytokine release?
    - Desired in cancer indications

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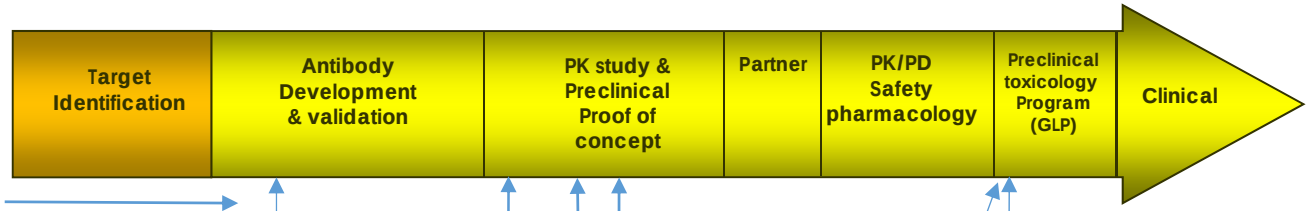
Recommended safety evaluations – Antibody projects



Target safety assessment

Antibody specificity:
-Homology searches
-Fc part should not bind cellular FcR receptors

In silico tests of candidate antibodies for Immunogenicity*

In vitro: 1. FcR binding in ADCC/CDC
2. T-cell dependent Immunogenicity (ADA)
3. Cytokine release assay.

In vivo: In-life and cytokine measurements (optional)
Plasma clinical chemistry and hematology.
Project specific: organ histology and soluble biomarkers

T-dependent ab response (TDAR)

Tissue cross-reactivity studies

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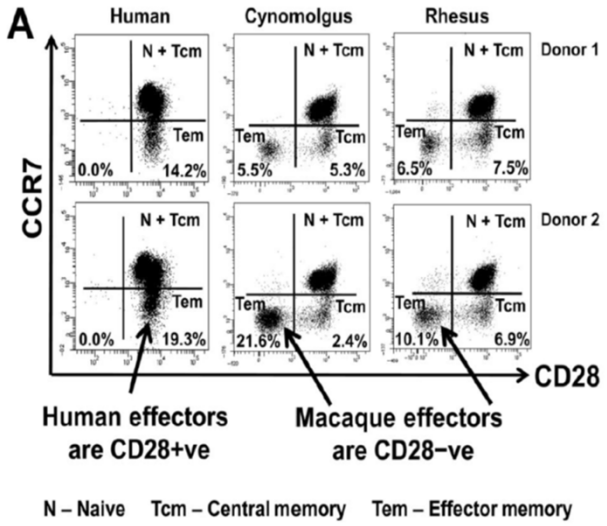
Clinical example: TeGenero

- First clinical trial with TGN-1412, in 2006
  - Humanised mAb specific for the CD28 receptor on T lymphocytes.
  - Aim: to expand the T-cell pool in patients with an imbalance (chronic B-cell leukemia, MS, rheumatism.
- No toxicity in preclinical toxicity studies
- No immunogenicity or immunotoxicity
- No activation of cynomolgous (monkey) T cells

TeGenero, con't.

- 1/500 of the NOEL in monkeys was given to six healthy male volunteers (one after another….)
- A few minutes after a single injection:
  - Nausea, headache, back pain, unconsiousness and collapse of large organs (e.g. liver).
- Fast reaction caused by massive lymphocyte activation and cytokine release ("cytokine storm")
  - i.e., a target-related effect, no ADAs etc involved
- Species differences in CD28 expression on sub-population of T cells
  - Non-relevant species..?



5 of the 6 men recovered, the 6th had to amputate fingers and toes.

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